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VITAMIN C CONTENT IN ORANGE JUICES OBTAINED BY DIFFERENT METHODS

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ABSTRACT

Introduction Proper nutrition has the task of providing the right amount of energy and nutrients. A balanced diet should include vegetables and fruit at every meal. These products are rich in vitamin C, which is involved in many metabolic pathways in the body. A well-balanced diet will generally provide you with enough ascorbic acid. The content of vitamins in products

depends on many factors, including the place of origin of the product, season, species, processing methods, or the conditions and time of storage. Vitamin C is easily destroyed by contact with oxygen, light radiation or metal ions. Aim of the study The aim of the study is to assess the content of vitamin C in juices squeezed from different types of oranges using various processing methods. **Material and methods** Three species of oranges were used for the study: Valencia, Saluciana and Novelina. Each type of orange was squeezed with 3 processing methods, using: a juicer, a slow-speed squeezer and a manual squeezer. The determination of vitamin C content in the obtained juices was performed by the Tillmans titration method with the use of standard, blue, 2,6-dichlorophenolindophenol dye. The vitamin C content in juices was determined: immediately after squeezing the juice, one hour after squeezing and 24 hours after squeezing. The juices were stored in the refrigerator between determinations. A pH meter was used to measure the pH, and a portable digital sucrose refractometer was used for the% sucrose measurement. **Results** The highest content of vitamin C immediately after pressing was observed in the juice of hand-squeezed oranges from the Salustiana variety. The decrease in vitamin C after 24 hours averaged 37.8%. Statistically significant differences were found between the method of squeezing and the time after squeezing the juice. Valencia variety was characterized by the highest pH, Navelina - the lowest. Significant differences were found in the mean level of the pH indicator and the type of orange.
Keywords: vitamin C, orange, juice, processing methods

INTRODUCTION

Vitamin C, also called ascorbic acid, occurs naturally primarily in all kinds of fruits and vegetables, sometimes it can be found in products of animal origin, although most often these are only trace amounts [1,2]. This vitamin is involved in many important metabolic pathways taking place. However, it is not synthesized in the human body (lack of L-gulonolactone oxidase enzyme), so it must be supplied with food. However, a well-balanced diet generally provides sufficient ascorbic acid with food [1-3]. Vitamin C is one of the most popular antioxidants. Due to its antioxidant properties, this vitamin is responsible for protection against cardiovascular disease and also protects body cells from oxidative stress [2]. However, ascorbic acid is not the only and best antioxidant. Among the potent compounds showing antioxidant activity we can also include carotenoids, especially astaxanthin, lycopene, lutein and β-carotene. These compounds are characterized by high activity against both reactive oxygen species and free radicals. Comparing β-carotene, astaxanthin and lycopene to other antioxidants (e.g. vitamin C) it can be stated that these compounds show higher antioxidant activity e.g. against singlet oxygen. Astaxanthin has also been shown to be a more potent antioxidant compared to β-carotene, vitamin E and vitamin C by 54, 14 and 65 times, respectively [4].

Vitamin C is absorbed by the body in about 70-80%, mainly in the duodenum and proximal part of the small intestine. The natural form of this vitamin is better absorbed from the gastrointestinal tract, as well as more effective in its action, compared to its synthetic counterpart (it reaches the required concentration in the body faster and maintains it for a longer period of time). The synthetic form of ascorbic acid is used by the body only in 30-40%. In the natural environment it occurs together with carotenoids, flavonoids and phenylacids, which makes this vitamin much more stable. The stability of vitamin C is also influenced by the presence of anthocyanins, where their small addition protects against oxidation, even at high temperatures. The course of the absorption process depends, among others, on absorption disorders, the use of certain drugs, or the occurrence of vomiting [2, 5]. Vitamin C has a strong antioxidant effect. It acts directly as an antioxidant. It also helps in the restoration of other antioxidants. In addition, this vitamin enhances the antioxidant effects of polyphenols such as flavonoids [6]. In determining the oxygen free radical absorbance capacity, ORAC (Oxygen Radical Absorbance Capacity) tables can be used. This is a measure of antioxidant capacity, which is determined for foods characterized by their antioxidant content. It was developed at U.S. government laboratories in Bethesda by the National Institute on Aging. ORAC values for various products are converted per 100g serving. It is suggested that consumption of foods with high ORAC definitely increases the antioxidant value [7]. Consumption of certain vegetables and fruits is associated with increased plasma antioxidant counts. It is recommended to consume foods high in antioxidants with each meal to prevent periods of postprandial oxidative stress [8].

Ascorbic acid is an extremely biologically active compound and is involved in many reactions and transformations that occur in the human body. The contemporary role of vitamin C includes, among others, participation in the biosynthesis of carnitine, collagen, synthesis of hormones, transmitters, as well as participation in fat and steroid metabolism. Increasing the bioavailability of calcium and non-heme iron in the human diet is also an important function of this compound [9].

The role of vitamin C is also associated mainly with the prevention of scurvy, which was first mentioned in antiquity. However, this disease is primarily associated with long sea voyages, during which access to fresh fruits and vegetables and thus to a source of vitamin C is difficult. Nowadays the cases of scurvy are sporadic and involve mainly the pediatric group

[10-12] There is also a popular claim that vitamin C prevents colds, which is not true. Yes, this compound has an effect on reducing the duration of the common cold, but it has no effect on their incidence in the majority of the population [13,14].

It is thought that high-dose vitamin C may have virucidal effects because it inactivates viral multiplication in vitro. Thus, administration of high-dose vitamin C may reduce the risk of developing cytokine storm in COVID-19 infection. It plays a key role in the treatment of SARS-CoV-2 infection in both home and hospital settings, leading to a beneficial effect in patients with mild symptoms as well as in patients with severe pneumonia. Therefore, it is recommended to add vitamin C to the national treatment guidelines for COVID-19 [15].

The nutritional value of food products, including the content of minerals and vitamins, depends on many factors, such as the place of origin of the product (e.g. region, country), the type of cultivation (organic/conventional), season, species, variety, methods of preservation, processing, or conditions (e.g. type of container) and time of food storage. Culinary processes are of particular importance in households. An unfavorable influence was observed on the content of water-soluble vitamins water-soluble vitamins (especially folates and vitamin C), were observed to be adversely affected by factors such as the type of culinary processing used, duration of processing, or temperature. During the initial food processing (slicing, shredding, grinding) losses of vitamin C can reach 20%, and during thermal processing (cooking) even up to 50%. Losses of vitamin C are also observed when meals are heated or defrosted too quickly. Vitamin C is also easily destroyed under the influence of food preservation or exposure to air and light. The degradation of ascorbic acid is also affected by the presence of metal ions, e.g. iron, copper [5,16-19]. In the case of juices available on store shelves, manufacturers often decide to add synthetic l-ascorbic acid to supplement the losses of its natural counterpart, as well as to protect other bioactive compounds (polyphenols, carotenoids) from oxidation, during production [18]. According to the Regulation of the Minister of Agriculture and Rural Development of September 30, 2003, fruit juices, fruit juices reconstituted from concentrated fruit juice, concentrated fruit juices, fruit juice powders, fruit nectars and fruit juices made with water extraction can be distinguished in the trade [20]. In addition to commercially available juices, consumers can opt for home-made juice using common household equipment such as a juicer. Commercial juices and home-made juices differ in terms of nutritional value, e.g. home-made juices have higher concentrations of biologically active compounds and higher antioxidant activity, compared to commercial juices [21]. Commercial juice production, on the other hand, uses different technologies used in the processing industry, of which the quality of the resulting juice strongly depends on the processing technology used (e.g. sedimentation instead of centrifugation preserves antioxidants in the juice, while the vacuum extraction technique produces juice with a high content of thermolabile, biologically active substances). Depending on the type of juice (e.g. clear/cloudy), different production techniques are used [22,23].

The predominant part of the market in Poland are clear juices and nectars, which are obtained from FC (from concentrated fruit juices) - however this does not apply to citrus fruits [24]. NFC juices (pressed/freshly squeezed juices) are also becoming more and more popular on the Polish market, although they are not as popular as in other countries. In Poland 2015, the NFC juice segment accounted for 4% of the value of juice sales, while, e.g. in France and the UK, this value represented 4% of the value of juice sales. In Poland in 2015, the NFC juice segment accounted for 4% of the value of juice sales, while, for example, in France and the UK this value represented 59% and 49% of the value respectively [25]. In terms of taste preference, apple, orange and blackcurrant juices are the most preferred by consumers [26].

The aim of the study was to estimate vitamin C content in juices obtained by different processing methods from different kinds of oranges available in Polish stores as well as to estimate sucrose content (%) and active acidity (pH) of these juices.

MATERIAL AND METHODS

Three types of oranges that were available in Polish stores in winter were used for the study: Valencia (country of origin - South Africa), Salustiana (country of origin - Spain) and Novelina (country of origin - Spain). Each fruit was washed, peeled, cut in half, and then squeezed using 3 processing methods: juicer, slow-revolution squeezer, and manual squeezer (made of metal).

From the squeezed juices, 5 ml of juice was taken into a 50 ml volumetric flask using a pipette. The flask was immediately made up to the mark with oxalic acid solution. The flask was then closed with a stopper and the solution was mixed. The sample thus prepared was allowed to stand in a dark place for about 5 minutes. After this time, the contents of the flask were filtered into a second volumetric flask.

10 ml of the filtrate was taken into a conical flask. The burette was filled with 2,6-dichloroindophenol solution. The samples were then titrated with the solution until a light pink coloration occurred lasting 10 seconds. The value obtained was recorded and then converted using the formula:

$$K = \frac{(V_0 - V_1)d}{m' V^2 m^0} \text{x} 100$$

K- amount of vitamin C in milligrams per 100 g of test sample

V_0 - amount of dye used to titrate the test sample, in milliliters

V_1 - the amount of dye solution used to titrate the solution in the blank, in millilitres

m^1 - dye titer, in milliliters per 1 mg of vitamin C

V_2 - amount of filtrate taken for titration in milliliters

d- capacity of volumetric flask, in milliliters

m_0 - weight of test material, in grams

The determination of vitamin C content in the obtained juices was performed by the titration method with the use of Tillmans dye (PN-A-04019:1998).

The determination of vitamin C content in juices obtained from 3 species of oranges using a hand squeezer, juicer and slow-release juicer was performed: immediately after squeezing, one hour after squeezing and 24 hours after squeezing. Juices were stored in a refrigerator at 6 °C between determinations. Each juice sample was analyzed in two parallel determinations, and the arithmetic mean of the obtained results was taken as the final result.

For measurement of active acidity (pH) a pH meter CPC 505 produced by Elmetron was used, and for sucrose measurements a portable digital refractometer HI 96801 produced by HANNA Instruments was used. The research was conducted in the laboratory of the Department of Human Nutrition of the Faculty of Dietetics of the Medical University of Silesia in Katowice.

Statistical calculations were performed using STATISTICA 13.0, Stat Soft Poland. Measurable data were characterized using ANOVA with repeated measures, univariate test, Friedman's ANOVA, one-way ANOVA and Kruskal-Wallis ANOVA for multiple related variables. Data were used for species and vitamin C content at a given time as well as for juice extraction methods and vitamin C content immediately after squeezing, after 3 hours, and after 24 hours. Count and multivariate tables were used for unmeasured data. The strength of the relationship was determined by determining Fi and Cramer's V. Statistical significance was determined at $p<0.05$.

RESULTS

The content of vitamin C in juices from the examined oranges is presented in Table 1. The highest content of vitamin C was observed in hand squeezed juice from oranges of Salustiana cultivar - just after squeezing (66.67 mg/100ml). This variety had the highest average amounts of this vitamin. No significant changes were seen in any sample after one hour. The greatest changes were seen only after 24 hours. It was also observed that the decrease in vitamin C after 24 hours averaged 37.8% with respect to the content of this vitamin immediately after squeezing.

Table I. Vitamin C content of the juices tested

SPECIES OF ORANGE	**Juice extraction method**	**Vitamin C content after squeezing (mg/100ml)**	**Vitamin C content after 1h (mg/100ml)**	**Vitamin C content after 24 h (mg/100ml)**
Valencia	Slow squeezer	40.91	39.39	39.39
	Juicer	46.97	45.45	19.70
	Hand squeezer	33.33	30.30	18.18
Salustiana	Slow squeezer	46.97	45.45	40.91
	Juicer	63.64	51.52	34.85
	Hand squeezer	66.67	53.03	39.39
Navelina	Slow squeezer	57.58	56.06	24.24
	Juicer	45.45	42.42	31.82
	Hand squeezer	60.61	50.00	30.30

Table II. Vitamin C losses by device

SPECIES OF ORANGE	Juice extraction method	Loss of vitamin C after 1h (%)	Loss of vitamin C after 24 h (%)
Valencia	Slow squeezer	3.72%	3.72%
	Juicer	96.76%	41.94%
	Hand squeezer	9.09%	45.45%
Salustiana	Slow squeezer	96.76%	87.10%
	Juicer	19.04%	45.24%
	Hand squeezer	20.46%	40.92%
Navelina	Slow squeezer	97.36%	42.10%
	Juicer	6.67%	29.99%
	Hand squeezer	17.51%	50.01%

Statistical significance between orange type and squeezing time was tested. No statistically significant differences were found (p=0.38) - ANOVA test with repeated measures. one-dimensional test. On the other hand, statistically significant differences were found between squeezing method and time after juice squeezing (p=0.005) - Fiedmann's ANOVA test (p<0.05).

Sucrose content (%) and active acidity values (pH) are shown in Table III. All the juices were characterized by an acidic reaction. The highest pH value was observed in Valencia orange juice squeezed with a slow speed squeezer (pH 1.96), while the lowest pH value was observed in Valencia orange juice squeezed by hand (pH 1.28). The Valencia orange variety had the highest pH indices. while Navelina had the lowest. The sucrose content of the tested oranges ranged from 10.4% to 12.73%. The highest sucrose content was observed in the juice from Valencia oranges squeezed in a juicer and the least from Salustiana oranges squeezed with a slow speed juicer.

Table III. Sucrose content and pH in the studied juices

SPECIES OF ORANGE	Juice extraction method	pH value	Content sucrose %
Valencia	Slow squeezer	1.96	12.10
	Juicer	1.82	12.73
	Hand squeezer	1.80	12.73
Salustiana	Slow squeezer	1.58	10.40
	Juicer	1.66	11.67
	Hand squeezer	1.40	11.03
Navelina	Slow squeezer	1.41	12.17
	Juicer	1.64	12.67
	Hand squeezer	1.28	11.90

Significant differences were found between the level of pH index and the type of oranges, p=0.025 (test - cross-section, simple ANOVA) and between the type of oranges and the amount of sucrose in the studied oranges - p=0.049 (Kruskal-Wallis ANOVA). On the other hand. there were no significant differences between the method of squeezing the juice and the content of pH index (cross-section test, simple ANOVA), as well as the method of squeezing and the content of sucrose (Kruskal-Wallis ANOVA test).

DISCUSSION

Orange juices are a rich source of vitamin C, which acts as an antioxidant in juices. Vitamin C concentration is an important indicator of orange juice quality and can serve as an indicator of product quality [27]. Citrus variety, fruit maturity, climate, juice processing and storage conditions affect the amount of vitamin C, sugar composition and other characteristic features of the juice [28].

Comparing our own results with the study conducted by Stankiewicz and Wieczorkiewicz [29] it was shown that the content of vitamin C differs depending on the kind of fruit. The material for the study consisted of one-day-old apple juices available in stores and home-made juices. The highest content of vitamin C was found in juices from supermarkets in which the producer indicated the addition of L-ascorbic acid. Regardless of the kind of juice and kind of fruit. the content of vitamin C decreased after 24 hours [29]. The same relation is observed in our study. The same relation is observed in our study. In every studied material the amount of this vitamin decreased after 24 hours. The highest decrease in vitamin C content was observed in the case of juice squeezed from Navelina oranges with a slow speed squeezer and it was 31.82mg/100ml. No noticeable change was observed after one hour.

Similar results to our own study were obtained by Lebiedzińska et al [30] in their study on vitamin C content in fruit juices and nectars from the stor, and freshly squeezed juices [30]. The richest source of vitamin C turned out to be fresh lemon juice, while the lowest amount of this vitamin was found in pear and apple juice. The value of vitamin C in fresh orange juice ranged from 24.43 to 25.12 mg/100ml, while in our study the level of this vitamin was higher, depending on the species, and ranged from 33.3 to 66.7 mg/100ml in freshly squeezed juice. The level of this vitamin was highest in Salustiana oranges squeezed with a hand squeezer (66.67mg/100ml) and lowest in Valencia oranges also squeezed with a hand squeezer (33.33mg/100ml). Thus, the level of vitamin C varied according to the method of preparation in our study and the species of oranges.

In a study by Djugan [31], differences in vitamin content were observed depending on the method of juice preparation. A higher concentration of ascorbic acid was shown in juice obtained by hand squeezing compared to juice prepared with a juicer. Similar results can be seen in our own study. This is probably due to thermal instability of vitamin C, which is reduced under the influence of heat generated by the juicer device. It is also possible that vitamin C is oxidized by contact with the cutting elements of the juicer, since trace amounts of iron catalyze the oxidation of ascorbic acid [31].

In a study by Mazurek and Jamroz [32] on the stability of vitamin C in blackcurrant juices during storage, blackcurrant juices and juices from other fruits The effect of 24 and 48 hours storage on vitamin C content was studied. The juices from oranges were characterized by low losses of L-ascorbic acid. Also, no significant differences were observed in vitamin C content between pureed and cloudy juices [32]. In our study, vitamin C levels changed differently depending on the type of orange and the squeezing method. The highest change was observed for Navelina orange juice squeezed in a slow juicer (31.82mg/100ml) and the lowest for Valencia orange juice also squeezed with a slow juicer (0.96mg/100ml). A similar result was obtained for Salustiana orange material squeezed with a slow speed squeezer.

A study by Chanson-Role et al [28] also measured vitamin C levels in orange juices. It showed that commercial juices contained significantly less vitamin (by about 15%) than homemade, which contained an average of 47.8 mg/100ml. similar to our own study, where the average level of this vitamin regardless of variety was 47.5 mg/100ml immediately after juicing. The difference between the amount of this vitamin in processed and non-processed juices may be due to vitamin C degradation that can occur during industrial production and commercial storage. As confirmed by the above study, in which the amount of vitamin C decreased by an average of 37.8% after 24 hours of storage in the refrigerator (30.98 mg/100ml) [28]. The study by Klimczak et al. also gave very similar results. In the analyzed juices the amount of vitamin C was: 40.85 mg/100ml and 36.15 mg/100ml [33]. One study [34] evaluated 14 commercial orange juices supplied by different manufacturers. It showed that these juices could contain from 15.0 to 22.4 mg /100 ml of juice [34].

Nabrdalik and Świsłowski's [35] study on microbiological evaluation of unpasteurized fruit and vegetable juices included determination of parameters such as pH. total acidity and relative density. The mean pH value of orange juice was 3.29. while in our study the pH value ranged from 1.28 to 1.96 depending on the orange species and squeezing method. Nabrdalik used commercially available juices for her study, whereas in our study the juices were freshly squeezed. You can see the differences in orange juice pH between the two studies, probably due to the different materials. It is assumed that a pH value that is too high may indicate dilution of the juice. while one that is too low suggests acidification of the juice [35].

Similar to the study of Silva et al [36]. where the pH level was 3.79. In our study, there was also a significant statistical relationship between the average pH level and the type of orange. This relationship was p=0.03. On the other hand, there were no significant differences between the way the juice was squeezed and the pH index value (p=0.54). The lowest pH was characteristic for Navelina orange juice from a manual squeezer and the highest for Valencia orange juice from a slow squeezer.

The carbohydrates found in orange juice are mainly glucose, fructose and sucrose. They account for 80% of the total soluble solids of orange juice. During processing and storage of orange juice, sucrose can be converted to glucose and fructose under acidic conditions. The average total sugar content is about 8.9g/100ml for orange juice. In a study by Zhang [37]. sucrose levels ranged from 3.3 to 5.3 g/100ml (average 4.4 g/100ml) [36]. In our study, the level was much higher, ranging from 10.4 - 12.73 g/100ml depending on the species immediately after fresh juice preparation. The sucrose content was also determined in a study by Lebiedzińska et al [38]. in which it was 3.14 g/100ml on average in fresh orange juice [38].

Vitamin C also works synergistically with quercetin (a flavonoid found naturally in nuts, fruits, and vegetables). Quercetin has a number of beneficial properties for the body; it is, among others. a strong antioxidant, it has anticoagulant, anti-inflammatory and antiviral properties (its action is associated with, for example, inhibition of proteases or binding of viral capsid proteins). It has been demonstrated that ascorbic acid increases the bioavailability of quercetin, and together with bromalin can form an effective complex in the prevention of COVID-19 [39, 40].

STRENGTHS AND LIMITATIONS

The analytical (titration) method is subject to researcher error. There are more accurate methods for measuring vitamin C. However. this study was designed to show differences between different orange species that might not be apparent in larger scale studies. The strengths of the present study are that the authors believe that vitamin C and its role in nutrition is marginalized and that there is little research on its content in products and studies on its intake are evaluated. In addition, the work has an important practical dimension because it gives information to the consumer on which technological processing method will be the best in terms of bioavailability of vitamin C.

CONCLUSIONS

1. Loss of vitamin C content in juices during storage was observed regardless of orange species and processing method.
2. There were statistically significant differences in vitamin C content according to the method of juice squeezing versus time after squeezing.
3. Oranges vary in vitamin C content. sucrose content and pH value depending on the species.
4. Vitamin C losses varied among different juicing methods.

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